

36. A vaccine against infection by brucellosis and/or a non-brucellosis disease, comprising a live attenuated *Brucella* host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which
5 DNA construct comprises:

- (i) a DNA fragment operably linked to a first promoter recognizable by *Brucella*, and encoding a heterologous antigen, and
- (ii) a complementation DNA fragment which is operably linked to a
10 second promoter recognizable by *Brucella*, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

37. The vaccine of claim 36, wherein the *Brucella* host cell comprises a *Brucella*
15 DNA fragment containing a stable non-reverting deletion mutation, having the nucleotide sequence of SEQ ID NO: 1 modified to delete nucleotides from position 1067 to position 1671.

38. The vaccine of claim 36, wherein the *Brucella* host cell is *Brucella*
20 *melitensis*.

39. The vaccine of claim 36, wherein the *Brucella* host cell is WRRP1, having ATCC accession number PTA-3753.

25 40. The vaccine of claim 39, wherein *Brucella* host cell WRRP1 has no antibiotic resistance markers.

41. The vaccine of claim 36, wherein the *Brucella* host cell is WRR51, having ATCC accession number PTA-3754
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NOTED ITEM(S) pg 29 - 35 claims

42. The vaccine of claim 41, wherein *Brucella* host cell WRR51 has no antibiotic resistance markers.

43. The vaccine of claim 36, wherein the promoter is a *Brucella* promoter.

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44. The vaccine of claim 36, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, *Yersinia pestis* F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, *Plasmodium berghei* antigens, *Plasmodium falsiparum* antigens, *Plasmodium*
10 *vivax* antigens, *Plasmodium malariae* antigens, *Francisella* antigens, staphylococcal and streptococcal enterotoxin fragment antigens; *Burkholderia* antigens, *Coxiella* antigens, *Clostridium* epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, *Helicobacter* antigens, *Borrelia*
15 antigens, *Legionella* antigens, *Bartonella* antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.

20 45. The immunogenic composition of claim 44, wherein the anthrax antigen is selected from the group consisting of *Bacillus anthracis* protective antigen and inactive variants of Edema Factor and Lethal Factor.

46. The immunogenic composition of claim 44, wherein the malaria antigens are
25 CSP and MSP1 antigens of *Plasmodium berghei*, *Plasmodium falsiparum*, *Plasmodium vivax*, or *Plasmodium malariae*.

47. The vaccine of claim 36, wherein the complementation DNA fragment comprises the *wboA* gene.

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48. The vaccine of claim 47, wherein the *wboA* complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

49. The immunogenic composition of claim 48, wherein when the vaccine is administered to a vaccinee, the lipopolysaccharide O-sidechain polysaccharide is produced in vivo and an antibody to the lipopolysaccharide O-sidechain polysaccharide is produced by the vaccinee in response.

50. A recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a promoter recognizable by *Brucella*, and
- (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

51. The recombinant DNA construct of claim 50, wherein the complementation DNA fragment comprises the *wboA* gene.

52. A recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a DNA fragment operably linked to a first promoter recognizable by *Brucella*, and encoding a heterologous antigen, and
- (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by *Brucella*, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in a host cell transformed therewith.

53. The recombinant DNA construct of claim 52, wherein the complementation DNA fragment comprises the *wboA* gene.

54. The recombinant DNA construct of claim 52, wherein the heterologous antigen is selected from the group consisting of anthrax antigens, *Yersinia pestis* F1 and V antigens and F1-V fusion proteins, malaria circumsporozoite and merozoite antigens, *Plasmodium berghei* antigens, *Plasmodium falciparum* antigens, *Plasmodium vivax* antigens, *Plasmodium malariae* antigens, *Francisella* antigens, staphylococcal and streptococcal enterotoxin fragment antigens; *Burkholderia* antigens, *Coxiella* antigens, *Clostridium* epsilon toxoids, botulinum toxoids, smallpox antigens, mycobacterial antigens, cancer antigens, HIV antigens, tetanus toxoids, diphtheria toxoids, pertussis toxoid, *Helicobacter* antigens, *Borrelia* antigens, *Legionella* antigens, *Bartonella* antigens, vaccinia antigens, antigen-GFP fusions, tagged antigens 6his and V5, fusions of antigens to secretory signals, and genes encoding therapeutic molecules or enzymes producing therapeutic molecules.

55. The immunogenic composition of claim 54, wherein the anthrax antigen is selected from the group consisting of *Bacillus anthracis* protective antigen and inactive variants of Edema Factor and Lethal Factor.

56. The immunogenic composition of claim 54, wherein the malaria antigens are CSP and MSP1 antigens of *Plasmodium berghei*, *Plasmodium falciparum*, *Plasmodium vivax*, or *Plasmodium malariae*.

57. A host cell transformed with a recombinant DNA construct of claim 50.

58. A host cell transformed with a recombinant DNA construct of claim 52.

59. A method for inducing protective immunity to brucellosis in a mammal comprising the step of administering to a mammal a vaccine comprising a live *Brucella* host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell will not exhibit full

virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a promoter recognizable by *Brucella*, and
- 5 (ii) a complementation DNA fragment which is operably linked to the promoter and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

60. The method for inducing protective immunity of claim 59, wherein the
10 complementation DNA fragment comprises the *wboA* gene.

61. The method for inducing protective immunity of claim 60, wherein the *wboA* complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

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62. The method for inducing protective immunity of claim 59, wherein when the vaccine is administered to a vaccinee, the lipopolysaccharide O-sidechain polysaccharide is produced in vivo and an antibody to the lipopolysaccharide O-sidechain polysaccharide is produced by the vaccinee in response.

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63 A method for inducing protective immunity to brucellosis or a non-brucellosis disease, or both, in a mammal comprising the step of administering to a mammal a vaccine comprising a live *Brucella* host cell having a rough phenotype, which host cell is sufficiently attenuated that upon exposure to a mammal the host cell
25 will not exhibit full virulence of non-attenuated *Brucella*, wherein the host cell is transformed with a recombinant DNA construct replicable in *Brucella*, which DNA construct comprises:

- (i) a DNA fragment operably linked to a first promoter recognizable by *Brucella*, and encoding a heterologous antigen, and

- (ii) a complementation DNA fragment which is operably linked to a second promoter recognizable by *Brucella*, and which complements a rough-conferring mutation in the host cell, thereby effecting a smooth phenotype in the host cell.

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64. The method for inducing protective immunity of claim 63, wherein the complementation DNA fragment comprises the *wboA* gene.

65. The method for inducing protective immunity of claim 4964 wherein the
10 *wboA* complementation DNA fragment encodes a peptide required for lipopolysaccharide O-sidechain synthesis.

66. The method for inducing protective immunity of claim 65, wherein when the vaccine is administered to a vaccinee, the lipopolysaccharide O-sidechain
15 polysaccharide is produced in vivo and an antibody to the lipopolysaccharide O-sidechain polysaccharide is produced by the vaccinee in response.

67. The method for inducing protective immunity of claim 63, wherein following administration of the vaccine to the vaccinee, the DNA construct is gradually
20 separated from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly cleared from the vaccinee.

68. The method for inducing protective immunity of claim 59, wherein following administration of the vaccine to the vaccinee, the DNA construct is gradually
25 separated from the *Brucella* host cell, whereupon the *Brucella* host cell reverts to a rough phenotype that is rapidly cleared from the vaccinee.

69. DNA construct pGSG5.